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SHELLFISH SANITATION RESEARCH. PROCEEDINGS OF THE 1959 PLANNING CONFERENCE

C. B. Kelly

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1960

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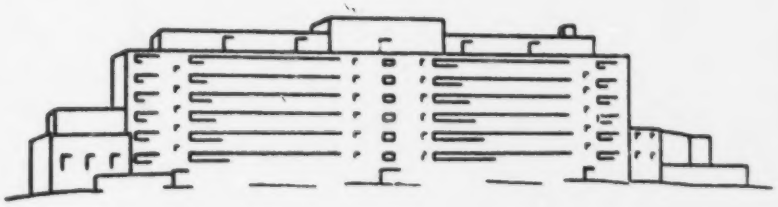

SHELLFISH SANITATION RESEARCH

Proceedings of the 1959 Planning Conference

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SHELLFISH SANITATION RESEARCH

Proceedings of the 1959 Shellfish Sanitation Research Planning Conference at Purdy, Washington, July 2-3, 1959, sponsored by the Milk and Food Research Program of the Public Health Service to determine research needs in shellfish sanitation in the Pacific Coast Area.

Edited by
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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services
Division of Engineering Services

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1960

I

PREFACE

Late in 1958, the Shellfish Sanitation Laboratory moved to Purdy, Washington to undertake research in problems in the sanitary control of shellfish in the Pacific Northwest. The principal objectives of the laboratory during the anticipated stay of five years are to conduct laboratory and field investigations to furnish scientific data necessary for the development of more efficient control measures and the development and evaluation of reliable standards of sanitary quality of the product at the various levels of production.

This report serves as a record of the transactions at a conference held on July 2 and 3, 1959 to consider the needs for research in shellfish sanitation in this area and to develop a program of investigations which would satisfy those needs. However, by including the notes prepared for the conference by the laboratory staff, it further records the background and general objectives of the laboratory as well as the accomplishments during the first eight months of activity.

We are deeply grateful to the conferees for their frank discussions of the problem. In many instances their participation extended beyond attendance at the conference. The proposed program of activities was developed after consultation with experts in the many fields related to sanitary control of shellfish. The fact that these include personnel of State agencies, Federal agencies, both in this country and Canada, Universities, and Industry is a clear example of the spirit of cooperation that prevails in all phases of sanitary control of shellfish in this country.

C. B. Kelly

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AGENDA

Thursday, July 2, 1959

9:00 - 9:30 A. M. Registration and Inspection of Laboratory

9:30 - 12:30 A. M. REVIEW OF SHELLFISH SANITATION PROBLEMS ON THE WEST COAST

Moderator: Bernard Bucove, M. D., Director of Health
Washington State Department of Health

9:30 - 9:45 A. M. Opening Remarks: Dr. Bucove

9:45 - 10:15 A. M. Responsibilities of the Public Service in Sanitary Control of Shellfish:

E. T. Jensen, Chief, Shellfish Sanitation Section, Milk & Food Program, Division of Engineering Services Public Health Service.

10:15 - 10:45 A. M. Scope of Problems in Shellfish Sanitation:

Dr. Keith H. Lewis, Chief, Milk & Food Research Sanitary Engineering Center, Public Health Service

10:45 - 11:15 A. M. Problems as Seen by a State Official:

Emil C. Jensen, Chief, Division of Engineering and Sanitation, Washington State Department of Health.

11:15 - 11:45 A. M. The Interests of the Shellfish Industry:

T. R. Ingham, M. D., President, Olympia Oyster Co., Olympia, Washington.

11:45 - 12:00 A. M. General Discussion:

12:30 Lunch

2:00 - 5:00 P. M. PROPOSED PROGRAM OF RESEARCH ACTIVITIES IN SHELLFISH SANITATION

Moderator: T. R. Ingham, M. D., President, Olympia Oyster Co., Olympia, Washington.

Proposed Program of Activities For Shellfish Sanitation Laboratory:

C. B. Kelly, Chief, and Staff, Shellfish Sanitation Laboratory.

1. History, Background and General Objectives
2. Background Data Collected by the Laboratory since November, 1958
3. Outlines of Proposed Research Activities

Studies on Shellfish Toxicity:

E. F. McFarren, Chemist, Milk & Food Research, Sanitary Engineering Center.

6:30 P. M. Dinner and Social Hour
Hotel Winthrop, Tacoma

Friday, July 3, 1959

9:30 A. M. DEVELOPMENT OF PROGRAM OF SHELLFISH SANITATION RESEARCH

General Discussion of Proposed Program

Moderators: E. T. Jensen and Dr. K. H. Lewis

MINUTES OF THE CONFERENCE

First Session*

REVIEW OF PROBLEMS

Moderator: Dr. Bernard Bucove

INTRODUCTION AND WELCOME

The conference convened at the Shellfish Sanitation Laboratory Purdy, Washington at 9:30 A. M. Dr. Bernard Bucove, Director of Health, Washington State Dept. of Health, opened the meeting and acted as moderator for the first session. Dr. Bucove welcomed the group on behalf of the Public Health Service and the Washington State Department of Health. He noted that all States on the West Coast including Alaska were represented and that the delegations included not only representation from Health Departments, but also from Fisheries Management Units and from Universities. He also noted representation from the U. S. Fish and Wildlife Service, the Division of Sanitary Engineering and the Food and Drug Directorate, Canadian Department of National Health and Welfare, and the British Columbia Department of Health.

He then presented a brief discussion of the participation in the program of shellfish sanitation research by the State Department of Health. An appropriation of $40, 000. 00 was passed by the 1957 State Legislature to acquire a site and construct buildings for the Shellfish Sanitation Laboratory. The State has provided the buildings and the maintenance of them including utilities and furnishing the services of a custodian. The Public Health Service provides the furniture and the equipment and the research personnel. Dr. Bucove also indicated that the State is collaborating in the research activities by cooperative field work undertaken by the two State Shellfish Sanitarians. Dr. Bucove then called the attention of the participants to the notes and proposed program of research studies prepared by the staff of the Shellfish Sanitation Laboratory. He asked for a frank and open discussion of the proposed program so that the conference could consider their ideas on the needs and methods of approach in shellfish sanitation research on the Pacific Coast.

RESPONSIBILITIES OF THE PUBLIC HEALTH SERVICE

Dr. Bucove then called upon Mr. Eugene T. Jensen, Chief, Shellfish Sanitation Section, Milk & Food Program, Division of Engineering Services, Public Health Service. Mr. Jensen presented a talk on the Responsibilities of the Public Health Service in Sanitary Control of Shellfish. An outline of the discussion prepared by Mr. Jensen is included as Appendix A of these Proceedings.

In addition to the items indicated in the outline, Mr. Jensen also emphasized that the Public Health Service program of shellfish sanitation control is one which is continually being revised. Major meetings are held in Washington every two years to consider revision of the manuals of recommended practice. Local problems are discussed in regional seminars held in each of the major shellfish producing areas. Seminars are held periodically in Boston, the Chesapeake Bay region, the Gulf Coast and on the West Coast. The Public Health Service intends to continue this cooperative approach, changing standards of criteria and operation to meet changes in industry practice. The regional seminars are held to adjust the manuals of operation to widely varying situations in the several geographical areas.

Mr. Jensen indicated that the purpose of this conference was to bring the group together to discuss the respective needs and emphasized that the notes and the proposed program of ac-

*Thursday morning, July 2, 1959.

tivities distributed to those present should be considered only as a reference point for discussions. The accomplishments of the Shellfish Sanitation Laboratory during the next five years are much dependent on a clear understanding of the problems and a well defined program of activities.

SCOPE OF PROBLEMS IN SHELLFISH SANITATION

Dr. Bucove then called on Dr. Lewis who digressed from the agenda to present a group award to the staff of the Shellfish Sanitation Laboratory. The award was presented to C. B. Kelly, W. Arcisz, M.W. Presnell and A. C. Sexton for superior work performance during their tour of duty at Pensacola, Florida. He noted that the Center had received many letters praising their efforts, and, as an example, read a letter from Dr. W. H. Aufranc, Regional Medical Director, Public Health Service, Region IV, expressing appreciation to the staff of the Shellfish Sanitation Laboratory for its work with the State Health Departments and the Regional Office. Messrs. Arcisz, Presnell and Sexton were given letters of commendation signed by Mr. Gilbertson and Mr. Hanson on behalf of the Public Health Service, and, in addition, were given cash awards. Mr. Kelly was presented a certificate of superior work performance.

Dr. Lewis then presented a discussion on the Scope of Problems in Shellfish Sanitation Research. The interest of the Public Health Service extends to all phases of the shellfish industry from growing through marketing and even to the way the product is served to the consumer. Sanitation problems are investigated not only in the laboratory but in the field and by means of collaborative studies with State authorities and industry. In conjunction with its research activities technical services are offered to State control agencies and industry in areas where special public health problems exist. He also indicated that we have tried not to stop at collecting information but rather to present this information to groups who could most use it. For this purpose a series of training courses in shellfish sanitation have been presented. He mentioned that the third such training course has been scheduled for Corvallis, Oregon, during the week of July 6. It will be held in the field to reach practicing shellfish sanitarians and industry members in this area.

The Public Health Service is very appreciative of the help that has been received in this highly cooperative venture. The Laboratory will continue to need help from many other groups, including industry and educational institutions as well as State laboratories. Dr. Lewis also mentioned that in addition to the work in Purdy on bacteriological problems, two other investigations pertaining to shellfish are in progress, both at the Sanitary Engineering Center. Studies in shellfish toxicity are conducted at the Center and in addition the Food Chemistry Unit is developing a test which will reveal the time-temperature history of shucked shellfish.

At the request of Dr. Lewis, Dr. R. P. Myers, Chief, Milk and Food Training, then presented a few remarks on the Milk & Food Training Program of the Center. He discussed the Shellfish Sanitation Course scheduled for July 6-10, 1959 at Corvallis and referred to the Training Program Bulletin describing courses in other phases of sanitation offered by the Sanitary Engineering Center.

PROBLEMS AS SEEN BY A STATE OFFICIAL

Dr. Bucove then called on Mr. Emil C. Jensen, Chief, Division of Engineering and Sanitation, Washington State Department of Health, who presented a discussion of Shellfish Sanitation Problems as Seen by a State Official.

Mr. Jensen first described the efforts of the Washington State Department of Health in bringing the Public Health Service Shellfish Sanitation Research activities to this state. He mentioned that members of his staff, particularly Mr. Ruppert and Mr. Girard worked with Public Health Service Shellfish Sanitation personnel on the selection of a suitable site for the laboratory and also outlined the efforts in justifying to the Legislature the need for an appropriation of $40, 000. 00 to provide the laboratory buildings and maintenance of the facility.

Mr. Jensen then presented a discussion of the status of sewage pollution and abatement in sections of the state adjacent to shellfish grow-

ing areas. He indicated that the problem of abatement of pollution was increasing but that there was encouraging progress in the installation of sewage treatment facilities in marine areas.

Mr. Jensen recognized that the State of Washington should assume primary responsibility for collaboration with the Shellfish Sanitation Laboratory in a comprehensive program of research, but entered a plea to other states to express their opinion on research needs and to indicate the extent to which they could participate in cooperative efforts. He pointed out that there was no question concerning the necessity for shellfish sanitation research in the Pacific Northwest. The species of shellfish are different than those in the East and Gulf Coasts, and there are vast differences in methods of growing and harvesting. As examples he mentioned the periodic exposure of shellfish to the air on oyster flats, diking for the cultivation of the native oyster and wet storage of shellfish in sink floats. He pointed to increasing problems in marketing practices.

Mr. Jensen also suggested the necessity for increasing the effectiveness and efficiency of the State program of shellfish sanitation control. With increasing numbers of problems in all areas of environmental sanitation, State Health Departments are now under increasing pressures, with little if any increases in staff. Thus, the State Official is continuously searching for more efficient methods of control.

Mr. Jensen presented a list of items he thought should be investigated at the Shellfish Sanitation Laboratory. This list as presented by Mr. Jensen follows:

1. Determine the indicator organism to be used in the sanitary evaluation of shellfish growing waters in this area.
2. Determine the populations of these organisms one would expect to find in safe shellfish producing waters.
3. At what rate do the various species of shellfish concentrate the indicator organism?
4. At what rate do they purify themselves of it?
5. What is the true sanitary significance of non-human fecality in shellfish and shellfish growing waters?
6. What are the seasonal variations in the feeding activities of the various species of shellfish?
7. How do such changes as salinity, water temperature, and turbidity affect the feeding activities of the various species of shellfish?
8. How do they affect the ability of the various species of shellfish to concentrate the indicator organism?
9. What is the die-out rate of selected pathogens in shellfish and shellfish growing waters?
10. How does air temperature affect the bacterial population of shellstock on exposed flats?
11. What is the sanitary significance of the artificial impounding of water over native oysters?
12. What is the sanitary significance of existing methods of wet-storage of shellstock?
13. What is the sanitary significance of dry-storage of shellstock?
14. How do the acceptable methods of shucking, packing, storing, and transporting shucked shellfish affect the bacterial populations of the final product?
15. Which laboratory procedures are best suited for the bacteriological examination of the various species of shellfish?
16. Under acceptable conditions, what is the anticipated storage life of the various species of shucked shellfish?
17. What should the bacteriological standards for shucked shellfish be?

In addition to bacteriological research such as mentioned previously, serious consideration should be given to research on the paralytic shellfish poison problem - information on the incidence and detection of the toxin in the various species of shellfish, the rate it is concentrated,

the identification of the causitive organism and the means of predicting its occurrence are necessary for a proper control program".

THE INTERESTS OF THE SHELLFISH INDUSTRY

Dr. Bucove then called on Dr. Ingham who presented a discussion of the interests of the Shellfish Industry. Dr. Ingham recognized that the Shellfish Laboratory was located in an area which offers many advantages for the cultivation of shellfish. He emphasized the importance of maintaining the sanitary quality of an area of such great potential. He indicated that other factors influence the productivity of this area and that the State Shellfish Laboratory at Quilcene was conducting studies on the influence of industrial wastes on the propagation and cultivation of shellfish. He made a plea for correlating the efforts of the two laboratories.

Dr. Ingham then pointed to a continually increasing competition for the use of marine areas by industry and the continually increasing population on the watersheds. He emphasized the importance of maintaining the sanitary quality of shellfish growing areas at the present high level since he felt that purification was not a satisfactory substitute for harvesting from approved areas. There are many in the shellfish industry who hold shore front property as a cushion against encroachment of populations in the areas immediately adjacent to shellfish growing areas and favored support of the sponsors of this movement by way of preferential taxes. Dr. Ingham expressed appreciation for help from the shellfish control agencies in preventing diseases and poisonings and also appreciated help in outbreaks. However, he strongly urged that only the particular violator be punished and that any publicity should not be adverse to the extent of persecuting those in industry who are operating at a high level of sanitation. It was his opinion that harvesting and shucking operations in this area resulted in the production of a good and safe product. He felt that the methods of transportation and marketing should be investigated because of the possibility of mishandling in transit. He indicated the need for some sort of an indicator dependable enough to show whether a good product had been mishandled.

Dr. Ingham indicated that there was a good potential for increase in production of shellfish in the waters of the State of Washington. He felt that with the increasing shortages of protein food products from the land, production of marine products would need to be increased.

GENERAL DISCUSSION

Dr. Bucove then opened the meeting for general discussion. He solicited comments by any of the conferees.

Mr. R. Bowering, Director, Public Health Engineering Service, British Columbia, Department of Health, suggested the need for studies on all of the commercially important species of shellfish. He also indicated that control of sanitation of clams in British Columbia was more difficult than that of oysters because clams are harvested in more remote areas. He thought that the problem with clams was more in handling than in the harvesting processes. Mr. Bowering also wondered whether there was any sanitary significance to the alleged influence by sulfite waste liquors on the feeding activity of shellfish.

Mr. Baker of Alaska indicated that at the present time the shellfish industry in Alaska was primarily in shrimp and crab but that there was a large resource of molluscs in the new state and they wanted to be prepared for the time when this resource might be exploited.

Mr. Greenberg of California pointed to a potential problem associated with the development of atomic power plants. He described the development of a reactor plant in Humboldt Bay area proposed for completion in 1962. Mr. Greenberg indicated that the State of California had completed a survey of the environment to obtain background information on radioactivity. Mr. Greenberg said that it was the first of a series of surveys to be conducted by the state until the reactor plant is finally installed. At that time the responsibility for monitoring would be assumed by the Pacific Gas and Electric Co. but the health department will continue with periodic checks. He indicated that up to the present they have determined only gross radioactivity. He recognized that such was not of as much value as other tests, but mentioned that he will enable determination of such elements

as Strontium 90 and other radioactive elements of public health significance.

Mr. Blair mentioned that the industry in Oregon was much smaller than in Washington, but the problems were similar. The pollution problem may be greater since the shellfish areas were in tightly enclosed bays almost completely surrounded by residential areas. He endorsed the list of problems outlined by Mr. E. C. Jensen and indicated a desire for information concerning the validity of enterococci as indicator organisms.

Mr. John Girard suggested the need for basic research in paralytic shellfish poisoning. A discussion on progress in this field then took place in which Dr. Quayle of the Pacific Biological Station endorsed the need for ecological work but pointed out that the extent of work in such studies appeared to be too large for any one facility to undertake. Mr. F. R. E. Davies described a sampling program in progress by the Food & Drug Directorate in collaboration with the Provincial Health Department which included routine testing of shellfish for toxicity.

Dr. Sparks of the College of Fisheries, of the University of Washington, mentioned that the institute has applied for a research grant for studies in shellfish toxicity.

John Dassow of the Fisheries Technological Laboratory, Seattle, Washington outlined two product quality programs in progress by the Fish & Wildlife Service. The first is a quality standards program which will ultimately result in the establishment of voluntary standards of quality and sanitation for all major fishery products, including frozen precooked and heat-and-eat products. In view of the activities of the Shellfish Sanitation Laboratory on molluscan shellfish, Mr. Dassow felt that the greatest interest of his group should be in shrimp and crab meat products. He also pointed out the necessity for the development of minimum standards for refrigeration facilities. The second program is an inspection service which will probably expand in the production of shrimp and crab meat in this area as a result of the gradual adoption of market standards by various consuming states. This is a voluntary program participated in by the industry with the Fish & Wildlife Service.

Mr. McMillin of the Olympia Oyster Co. suggested parallel studies in shellfish to determine relationships between indicator organisms and specific pathogens. He also indicated the need for training of operators of retail outlets and for those who handle the product in transit.

Mr. Pollack, Secretary of the Pacific Coast Oyster Growers Association endorsed the need for the development of improved methods of handling the product in transit and indicated that this problem existed in the entire fish industry as well as in the shellfish industry. He endorsed the idea of an educational program for dealers to inform them concerning proper methods of storage of the product and also the need for a test to detect mishandling. Dr. Lewis again referred to the work in progress at the Sanitary Engineering Center on a chemical test for freshness of oysters and indicated that it might have practical application in the problem mentioned by Mr. Pollack.

Second Session*

PROPOSED PROGRAM OF RESEARCH ACTIVITIES

Moderator: T. R Ingham, M.D., President Olympia Oyster Company

SHELLFISH SANITATION

Dr. Ingham called on Mr. Kelly to present the proposed program of activities for the Shellfish Sanitation Laboratory. Copies of the proposed studies together with notes on the history and general objectives of the Shellfish Sanitation Laboratory and background information collected at Purdy since November 1958 were distributed. Mr. Kelly pointed out that the outlines of proposed research activities were developed after consultation with Shellfish Sanitation Control Agencies in the three Pacific Coast States and British Columbia as well as with shellfish biologists in these same areas. He indicated these outlines should be considered only as a basis for discussion which he hoped would be held on each one of the projects. He also pointed out that the five proposed projects were closely related and integrated and that all supported and supplemented each other. He also noted that all of the items in the list presented by Mr. E. C. Jensen were included in the proposed projects. A copy of the material was distributed and is included in these proceedings as Appendix B.

Mr. Kelly then read from these notes. At the end of his presentation he indicated that he would appreciate a study of the material by the group in preparation for discussions to be held on Friday morning.

STUDIES ON SHELLFISH TOXICITY

Dr. Ingham then called on Mr. McFarren for a discussion of studies in shellfish toxicity. Mr. McFarren pointed out that the method of prevention of outbreaks of shellfish poisoning involves the identification of the presence of the toxin in the food by a laboratory assay. He indicated that a program of monitoring the producing areas had been in progress on both coasts, but in spite of this two outbreaks occurred in 1957. Oysters were involved in the outbreak in British Columbia This is the first recorded association of oysters with shellfish poisoning in this area.

At the present time the accepted technique for assaying shellfish poison is a biological procedure. The method was adopted at the October, 1958 meeting of the Association of Official Agricultural Chemists as an "Official Method". As a result of this improvement in the method, which also made possible the determination of the micrograms of poison in a known amount of shellfish, and the collection of recent epidemiological data, it has been established that as little as 1 milligram of the poison may cause fatalities in humans. As little as 0.1 mg. caused severe illness in a small child during the 1957 outbreak in New Brunswick. However, these and other studies indicate that improvements in the assay are still needed.

A chemical method of analysis for paralytic shellfish poison has been developed and under the auspices of the Association of Official Agricultural Chemists is now being studied collaboratively.

Other basic studies of the paralytic shellfish poison problem are needed. These include:

a. A determination of how many plankton species produce the toxin associated with shellfish. (Are there others than Gonyaulax?)

b. Methods for the purification or detoxification of poisonous shellfish. (Rate of detoxification may vary with the species.)

*Thursday Afternoon, July 2, 1959

c. Studies on the ecology of the causative organisms as well as the shellfish.

d. The chemical structure of the poison needs to be further investigated.

e. The exact site of action in the poisoned organism is unknown and no effective treatment against the poison is known.

Mr. McFarren then informed the group of four publications of the Sanitary Engineering Center on the subject of shellfish toxicity, these are:

1. Association of Official Agricultural Chemists "Official Method" of Analysis. The Bioassay of Shellfish for Paralytic Shellfish Poison. J. Assoc. Offic. Agr. Chemists 42:37, (February, 1959).

2. McFarren, E. F., "Report on Collaborative Studies of the Bioassay for Paralytic Shellfish Poison", J. Assoc. Offic. Agr. Chemists, (May, 1959).

3. McFarren, E. F., Schantz, E. J., Campbell, J. E., and Lewis, K. H., "A Modified Jaffe Test for the Determination of Paralytic Shellfish Poison", J. Assoc. Offic. Agr. Chemists, (May, 1959).

4. McFarren, E. F., Schafer, M. L., Campbell, J. E., Lewis, K. H., Jensen, E. T., and Schantz, E. J., Public Health Significance of Paralytic Shellfish Poison. A revision and enlargement of an article in "Proc. Nat'l. Shellfisheries Assoc. 47, 114 (1956)" to be published in "Advances in Food Research" 10 (1960).

Third Session*

DISCUSSION OF RESEARCH PROGRAM

Moderators: K. H. Lewis and E. T. Jensen

EVALUATION OF BACTERIOLOGICAL METHODS

In opening the meeting, Mr. Jensen stated that the objective of the session was to obtain the ideas of the participants of the needs in shellfish sanitation research on the West Coast, and that the Public Health Service was anxious to have comments and ideas from the participants. He suggested that the projects be reviewed in detail one by one and that the order of presentation be changed to allow first a discussion of methods for the bacteriological examination of sea water and shellfish, particularly the tests which involved separation of the coliform group into its various types.

Mr. Kelly then presented a brief description of the methods for the enumeration of coliform organisms, E.C. gas positive organisms, and classification of coliform types by the IMVIC reactions. He described the liquid confirmatory procedure for the enumeration of E. coli Type I and other coliform types capable of growth with the production of gas at 44.5°C and mentioned that this portion of the coliform group had been accepted as the index organisms in the tentative market standards for Eastern oysters. He pointed out that the test did not define any one species, that it defined mostly E. coli type I, but that other coliform types occasionally produced a positive test. He gave a brief summary of the evaluation of the E.C. gas positive test by the Shellfish Sanitation Laboratory at Pensacola and at Purdy, and by others in collaborative studies in which several states on the Eastern Seaboard participated. He pointed out that with water samples, 95% of the E.C. gas positive tubes were found to contain E. coli type I and approximately 80% of those from shellfish samples were found to contain this organism. With both water and shellfish samples the predominant coliform type other than E. coli type I was aerogenes type I. All of the organisms isolated from E.C. gas positive tubes were demonstrated to again grow at 44.5°C with the production of gas in pure culture.

Mr. Kelly deplored the lack of suitable reference methods for the evaluation of procedures for the enumeration of coliform types. He cited experiments at Pensacola which demonstrated a loss of up to 50% of E. coli type I when attempts were made to apply the procedure of streaking on EMB for the purpose of isolation of the various coliform types in pure culture. Pour plate methods were inefficient, both in quantitative recovery of the coliforms and the regularity with which coliforms produced typical colonies.

Because the E.C. gas positive test does not define E. coli type I, the term "fecal coliform organisms" has been applied. Mr. Greenberg suggested that the term had some undesirable features. Others entered into the discussion, agreeing that the term had disadvantages but could offer no recommendations for a substitute. Dr. Lewis indicated that if the term E. coli were used one would be technically in error, since positive tests are not limited to the species, E. coli, as defined taxonomically. However, one basically is attempting to develop a test which can demonstrate fecal pollution; therefore, he favored the use of "fecal coliform bacteria" to emphasize the idea of fecal pollution.

A discussion then took place on the potential application of the membrane filter method in sea water. Mr. Greenberg pointed out that, in addition to other disadvantages, the membrane filter procedure may not measure the same coliform flora as the multitube dilution methods, and Membrane Filter technic could not be applied to shellfish. He therefore doubted that it ever would arrive at the status of a complete substitute for the multiple tube method. The

*Friday morning, July 3, 1959.

group was then informed of comparative tests conducted at the Shellfish Sanitation Laboratory. Some 200 sea water samples have been submitted to comparative MF/MPN tests and the data is now at the Sanitary Engineering Center for statistical analysis.

Dr. Liston then brought up the point of phage typing of coliform organisms. He reported on some work in France on this method of identification. Mr. Greenberg pointed out that in his observations one came to the same conclusions as from routine coliform tests and that phage typing was much more tedious.

The group then considered methods for enterococci and the sanitary significance of these organisms.

Mr. Greenberg reported good results with the Slanetz Membrane Filter procedure and that the English Procedure and the Mallman Technic showed equivalent MPN values. Dr. Liston indicated good results with the multiple tube method in the examination of frozen foods and pointed out that coliforms seemed to die-off more rapidly than enterococci. Dr. Lewis observed that this difference should be of concern, since there might be a possibility of rejecting a product on the basis of tests for enterococci which should be considered safe from the standpoint of its content of pathogenic organisms.

Dr. Liston suggested consideration of plate counts at twenty degrees as well as at thirty-seven degrees centigrade. Twenty degree counts are useful in estimating the numbers of spoilage organisms in fishery products since the optimum and often even the maximum temperature of growth is below 37°C. He suggested the advantage of twenty degree counts in process samples for this reason. He also indicated that a change in the ratio of twenty to thirty-seven degree counts might be helpful in indicating points of contamination.

INVESTIGATIONS IN SHELLFISH GROWING AREAS

Mr. Jensen then suggested an evaluation of Project I, Evaluation of The Sanitary Significance of "Fecal Coliform Organisms" in Shellfish and Shellfish Waters. He described the development of the current tentative market standard for Eastern shucked oysters, noting that the present standards were the result of a series of collaborative studies undertaken by States and Cities on the Eastern and Gulf Coasts and the Shellfish Sanitation Laboratory. With recognition of these organisms in the new market standards, there came the question of the sanitary significance of the E.C. gas positive group of organisms and thus the reason for the development of a collaborative study to determine the association of these organisms in natural estuaries with known pollutional discharges.

Mr. Jensen outlined the general objectives of Project I, indicating relationships to a nationwide study to evaluate the sanitary significance of "fecal coliform organisms" in shellfish growing areas. The study involves comprehensive sanitary surveys in moderately polluted and clean shellfish growing areas of similar hydrography. He indicated that the plan of study involved a determination of the correlation between numbers of "fecal coliform organisms" found in these two areas with the quantity of fecal discharges in the estuary. He told the group that he and Mr. Kelly on recent trips had received endorsement of the project by five states on the Eastern Seaboard and one on the Gulf Coast. Mr. Kelly then pointed out that Project I was in progress; that one series of bacteriological samplings had been conducted; and that the sanitary survey conducted by the Washington State Department of Health was practically complete. He then reviewed the results of the bacteriological survey.

Mr. Jensen indicated that it would be desirable for state agencies on the West Coast to include such investigations in their routine sanitary surveys of growing areas. Mr. Blair of the Oregon State Department of Health advised that a limited amount of work might be done in Oregon and Mr. Greenberg indicated that as far as the laboratory was concerned, he would be willing to undertake some studies. Dr. Lewis felt that the Shellfish Sanitation Laboratory should assume responsibility for undertaking the project as outlined for the Interstate Cooperative study, collecting as much information as possible in the two areas in Washington. He also indicated the value of supplemental information collected by the states during the course of routine surveys of shellfish growing areas which might produce data on the correlation of coliform and E.C. gas positive results in areas of known sanitary condition.

Mr. Greenberg inquired whether surface or bottom water samples were collected. He was told that bottom samples were always collected at the Shellfish Sanitation Laboratory and that top samples were collected when conditions of stratification were observed. Stratification was detected by determination of salinity in the field, using the hydrometric method described in the U.S. Coast & Geodetic Survey, Special Publication #298*.

The bacteriological water sampler used by the Shellfish Sanitation Laboratory was demonstrated. The group was told that a sketch of the sampler was available on request. Some discussion also took place on the significance of examinations of bottom muds. The experiences of the Shellfish Sanitation Laboratory in Pensacola were described, mentioning that there was not a well defined method for expressing the results. At the present time the results are expressed by the Shellfish Sanitation Laboratory in terms of numbers of organisms per unit volume of mud, measured after allowing the sample to settle for 24 hours, and also per unit weight of dry solids. A bottom sampler used by the Shellfish Sanitation Laboratory was described. A sketch of this device is obtainable on request.

Recommendations

1. The group endorsed the idea of the sanitary survey study project and some indicated their desirability to participate to the limit of their facilities and work load.

2. The emphasis should be placed on coliform and E.C. tests.

3. Membrane filter procedures and methods for the enumeration of enterococci should also be investigated but should receive secondary consideration.

4. The suggestions of Dr. Liston on 20° counts should be followed.

ACCUMULATION AND ELIMINATION OF BACTERIA BY SHELLFISH

A flow diagram of the proposed laboratory set-up for these studies was presented. (This experiment is described in detail in Project III contained in Appendix C). The study would involve setting up three laboratory flats of shellfish each flat possibly containing two species. One flat would be maintained at a coliform level equivalent to that prevailing in a moderately polluted area (Median coliform MPN 70 to 700). Water flow would be continuous; pollution dosage would also be continuous. A second flat would be furnished water at a coliform level approximately 1/10 of the first, dilution being prepared from the first flat by continuous flow. The third flat would be a control, receiving the sea water only.

In the Pensacola experiments it was found that the variation in coliform content of the natural water was in excess of tolerances for good control and it was therefore found necessary to develop methods for the removal or destruction of coliform organisms. Ultraviolet ray treatment was found to be successful. Experiments at Purdy have indicated that this method is equally successful with these waters.

After establishment of equilibrium between the water and the oyster, samples of both water and oysters would be collected periodically for a period of about five days. At the end of that time all flats would be furnished coliform zero water to study the rate of elimination of bacteria by the shellfish. To obtain relationships between the laboratory observations and those under more natural conditions it was proposed to install a flat of shellfish in the estuary near the water intake and to sample the water and shellfish while the laboratory experiments were in progress.

A discussion took place on the water requirements of shellfish in a captive environment. From the discussions, it appeared that the proposed water flows per animal were ample. The group then discussed the significance of the constantly varying water flow per animal in the experiments, since the number of shellfish were constantly reduced as the experiment progressed. Mr. Kelly pointed out that in similar experiments

*Zerbe, W.B., and Taylor, C.B., "Sea Water Temperature and Density Reduction Tables." U.S. Coast & Geodetic Survey, Special publication #298.

in Pensacola this was of concern but that a review of the results indicated no significant change in the water/oyster coliform ratios as the experiment progressed and the animals were removed from the system. Dr. Quayle indicated that he thought the experiment as designed would not produce any adverse situations; that the matter of the changing quantity of water presented to the shellfish should not be of great concern. He mentioned that one should recognize that laboratory scale experiments cannot reproduce the natural environment and therefore endorsed the scheme of setting out a flat of shellfish in the natural estuary for comparison with results obtained in the laboratory. The general opinion was that methods mentioned in the outline to determine optimum water flow, particularly the bacteriological tests and measurements of fecal deposits at various locations in the tank, should provide the desired information.

It was suggested by Mr. Lindsay and Mr. Woelke that clams would probably respond more normally if they were placed in the tanks syphon end up. This would necessitate some means of support. The use of sand is undesirable because of difficulties in cleansing the tanks. Mr. Lindsay suggested a support of plexiglass, probably a trough-like arrangement in which the clams could be supported.

SURVIVAL OF ENTERIC ORGANISMS IN SHELLFISH AND SEA WATER

Sea Water Studies

A detailed description of the project was presented. A copy of the proposed project will be found in Appendix C of this report. Mr. Eugene Jensen then posed the question of the value of continuing with investigations on the relative survival of coliforms and enteric pathogens in sea water by procedures which had been followed in Woods Hole and Pensacola. Since the studies were conducted on relatively small volumes of water in bottles, the results might be more of classical interest that of direct practical application to natural situations. Mr. Greenberg was of the same opinion, pointing out that further work would be only repetition of a number of such studies undertaken in many parts of the country by many workers. Mr. Kelly indicated that this was true and he would have no objection to minimizing the amount of work done in sea water under such captive conditions. Dr. Lewis then pointed out that he agreed with these thoughts, but suggested a limited amount of work to determine the behavior of specific types of coliform organisms, and possibly enterococci as compared with enteric pathogens. He pointed to the newly proposed "fecal coliform" group, recent developments in methodology for enterococci, and the lack of comparative investigations with these two indicator organisms and enteric pathogens.

Discussion then took place on the possibility of tracing the die-off of pathogens and indicator organisms in a natural estuary. The discussion indicated that methods for the enumeration of salmonella in the small quantities present in natural waters were not suitably developed to warrant the initiation of such studies at this time but that recent developments in techniques might be considered for evaluation. Mr. Kelly mentioned the possible use of the membrane filter or filtration through diatomaceous earth filters for concentrating the organisms from large volumes of water. Both of these methods have been used on fresh water in England with some success. Mr. Greenberg indicated that the residues might be reconstituted to a known volume for application of the decimal dilution technic and thus an MPN could be obtained.

Recommendations on Sea Water Studies: It was proposed to follow the suggestions of Dr. Lewis by conducting studies to determine the relative behavior of the groups of organisms which he mentioned.

Studies on Shellfish

As indicated in the outline for Project IV these studies are designed to determine the relative behavior of specific pathogens and indicator organisms in shellfish stored under conditions simulating commercial practice. Mr. Kelly pointed out the difficulties in producing a situation in the laboratory in which shell stock would behave in a manner similar to that observed under commercial conditions. He also outlined the difficulties of polluting shellfish with salmonella. With these two points in mind he suggested that work on shell stock in the laboratory should be minimized. The situation with shucked shellfish however, is different. It is possible to contaminate shucked shellfish and store the product under conditions which would

produce behaviors similar to those observed in commercial practice. Mr. Jensen pointed out that there was far more commercial traffic in shucked shellfish than in shellstock, particularly of the Pacific and Olympa oyster, and thus studies on shucked shellfish would have greater commercial significance.

Recommendations for Studies on Shellfish: It was the consensus of the group that studies on shucked shellfish should be emphasized, anticipating that the relative behavior of indicator organisms and pathogens could be obtained from these studies and possibly later applied to observations on shell stock under commercial conditions. It was suggested that work with shell stock be done under commercial conditions. An outline of study for such investigations is described in Project V.

INVESTIGATIONS OF COMMERCIAL PRACTICES

The objective of the project is to determine the bacteriological changes in the successive stages of harvesting, processing and marketing of shellfish. This study is particularly important in the Pacific Northwest since many of the commercial practices are quite different than those in other parts of the country and thus the sanitary significance of some of these practices should be investigated. The wide spread use of sink floats for wet storage of oysters and other shellfish and the practice of storage of shellfish on barges are unique to this territory and little is known of the bacteriological changes taking place during such processes. The project suggests the examination of traced lots of shellfish sampled at key points in good commercial processing from the time of harvesting to marketing. This information is needed for the formulation of suitable market standards. Mr. Jensen again referred to the market standards for East Coast shucked oysters, mentioning that they were developed from results obtained in a succession of cooperative studies and indicated the need for similar cooperative investigations on the West Coast. Dr. Lewis pointed out that investigations of this sort would undoubtedly result in improvement of the quality of the product, since they would indicate stages in processing where the greatest potential for mishandling might exist. Dr. Liston again suggested low temperature counts. He indicated that this study would afford a good opportunity for correlation with work he was doing on the general bacteriology of fish and shellfish, thus another reason for parallel 20°C and 37° counts. Dr. Quayle indicated problems in maintaining good keeping qualities of shucked shellfish, particularly when packed in larger containers. Discussion also took place on problems in the shipment of shellfish. Many instances of spoilage were reported as the result of mishandling or at least of poor refrigeration practices while the product was in transit.

Mr. Jensen and Mr. Kelly both suggested that the accomplishments in this project would depend largely on cooperation with state and city shellfish control agencies: that data on handling up to the point of shipment could be obtained by the Shellfish Sanitation Laboratory, but we would have to depend largely on cooperation by other agencies for the collection and examination of samples as received at the market. Mr. Jensen pointed out again that this information was of vital need for the formulation of reasonable market standards. Mr. Kelly informed the group that preliminary discussions had been held with the states on the West Coast and that all had declared their intention to cooperate with such a project to the limit of their facilities and work load.

GENERAL DISCUSSION

After the individual projects were discussed, the chairmen then called on the group for remarks about the over-all program. Mr. Greenberg indicated that he thought the program was well rounded and could see no further suggestions other than those which had been made from the floor. Mr. Girard was of the same opinion. Mr. Emil Jensen also had no further comment but solicited comments on the distribution of minutes of this conference. The group indicated their desire for a Proceedings which might be distributed to the states and other agencies. Mr. Kelly offered to prepare a preliminary draft of the Proceedings to be submitted to each of the conferees for review of their individual comments prior to final publication as a Sanitary Engineering Center Publication. This idea met with the approval of the group.

The suggestion was also made that another meeting should be held in about a year to review the accomplishments of the laboratory. Mr. Emil Jensen suggested a meeting in the spring or summer of 1960 in advance of the scheduled Shellfish Sanitation Workshop and that the Public Health Service make a decision on a date for the meeting.

Appendix A

RESPONSIBILITIES OF THE PUBLIC HEALTH SERVICE IN SANITARY CONTROL OF SHELLFISH

E. T. Jensen

I. Why is the Public Health Service interested in sanitary control of the shellfish industry?

A. The PHS law directs the Service to cooperate with the States in the prevention of interstate spread of disease.

B. The Cooperative State-PHS-Industry program for the Certification of Interstate Shellfish Shippers provides effective sanitary control over a class of food products which have an unusual potential for transmission of disease.

1. Program is limited to oysters, clams and mussels.

2. State participation is voluntary. The requirements of the Food, Drug, and Cosmetic Act, as they relate to interstate shipment of shellfish, are not replaced.

3. Program is administered jointly by the States, the PHS, and the shellfish industry; however, many other Federal, State and private organizations also participate. Canadian shellfish production is included under the terms of a 1948 bilateral memorandum of agreement.

C. What are the reasons for this specialized food sanitation program?

1. Oysters, clams and mussels have a unique potential for the transmission of communicable disease.

a. These shellfish may grow in sewage polluted areas or may be contaminated with sewage during holding in storage areas.

b. These shellfish are plankton feeders and will concentrate any micro-organisms which might be present in the water.

c. They are frequently eaten raw or with only minor cooking.

d. The entire animal, including the contents of the stomach and intestinal tract, are eaten.

2. Shellfish may accumulate paralytic shellfish poison

a. Paralytic shellfish poison has never been found in Chesapeake Bay area; however, it is common in Canada, on the West Coast, and in some other parts of the world.

b. Paralytic shellfish poison may be seasonal or year around.

3. Shellfish may accumulate industrial wastes or radioactive materials which are poisonous to man.

II. Sanitary control of shellfish is based on control-of-source in contrast to other food control programs and which are based on plant sanitation and/or laboratory examinations.

A. Almost every instance of shellfish being involved in the spread of a communicable disease has been traced to sewage contamination of the shellfish growing or storage areas:

B. Control of paralytic shellfish poison and contamination of shellfish with industrial

wastes or radioactive materials is also based on control of source; however, objective examinations may be more effective in these areas.

C. Sanitary handling of shellfish subsequent to harvesting is also important since shellfish are a readily perishable food product.

III. The development of shellfish sanitation activities in the United States.

A. During the period 1900 to 1924 a number of the State Health Departments, the PHS, and the FDA made numerous attempts to provide adequate sanitary controls over the growing, harvesting, and processing of shellfish.

1. Implementation of State control programs.

2. PHS sanitary surveys of a major portion of the U.S. shellfish growing areas.

3. Official actions of PHS and FDA to prevent the interstate shipment of polluted shellfish.

a. FDA "Food Inspection Decision" of 1909 made it unlawful to ship or sell in interstate commerce any shellfish taken from insanitary or polluted beds.

b. PHS amended interstate Quarantine Regulations in 1917 to prohibit the interstate transportation of oysters and clams grown or harvested under insanitary conditions.

B. Despite these efforts disease outbreaks due to polluted shellfish continued to occur.

1. During the winter of 1924-25 a major outbreak of typhoid fever developed in New York, Chicago, Washington and several smaller cities (1, 500 cases, 159 deaths).

2. The outbreak was associated with oysters and the resulting adverse publicity halted the sale of oysters.

IV. The Shellfish industry and several State health departments appealed to the Federal government to develop a system of sanitary controls which would prevent disease outbreaks due to sale of polluted shellfish and thereby reestablish public confidence in shellfish as a food.

A. The PHS sponsored a shellfish sanitation meeting in February of 1925 to review this situation. The meeting was attended by a number of State and city health officers, representatives of FDA, FEW and PHS, and the shellfish industry.

B. The framework for the cooperative shellfish sanitation program was developed at the meeting.

V. The Cooperative State-PHS-Industry Program for the Certification of Interstate Shellfish Shippers is voluntary in nature and is based on the principle that each of the interested groups must meet certain responsibilities.

A. The program recognizes the State responsibility for control of growing areas, and harvesting and packing practices.

B. PHS accepted the responsibility for development, in cooperation with State and industry, of uniform sanitary standards.

C. Development of adequate standards require adequate research.

1. Direct PHS research.

2. Cooperate PHS-State research.

3. State research.

4. PHS supported research.

D. PHS also evaluates State shellfish sanitation programs to determine that standards are being met, and publishes a list of shellfish shippers certified by the States.

E. Industry is a partner in all phases of the program.

VI. Although numerous technical changes have been made in the certification program over

the years, the basic control concepts are substantially unchanged from those adopted in 1925.

A. PHS Manual of Recommended Practice for Sanitary Control of the Shellfish Industry is revised about every ten years.

B. The certification program is administered through national shellfish sanitation workshops held in Washington, D. C., and through regional seminars held yearly in all major shellfish producing areas.

C. Program must be sufficiently flexible to apply to widely varying geographic and industry conditions.

VII. No immediate changes are contemplated in the organization or administration of the program.

Appendix B

HISTORY, BACKGROUND, AND GENERAL OBJECTIVES OF THE SHELLFISH SANITATION LABORATORY

Prepared by the SSL Staff

Sanitary control of shellfish in the United States is carried out as a cooperative program with the Public Health Service as the co-ordinator in close collaboration with the State Control Agencies and the Shellfish Industry. The states are directly responsible for the sanitary control of shellfish, and the Public Health Service, on evaluation of the adequacy of the state control endorses those programs which meet accepted requirements. This obviously requires standards of operation on which to base the adequacy of control by the states and it has therefore become necessary to develop manuals of recommended practice for harvesting, processing and marketing of shellfish for the use of the states and industry as guides of uniform, accepted practice.

The Shellfish Sanitation Laboratory acts as a research agency to investigate problems in shellfish sanitation necessary for the establishment of safe and reasonable control measures. Many of the recommendations in the manuals being, unique to the industry require evaluation before they can be accepted as reasonable as well as safe. In addition, standards of quality, bacteriological or otherwise, must be justified by investigations to determine their feasibility. It is in these areas of research that the Shellfish Sanitation Laboratory has been working.

The principal objectives of the laboratory can be summed up as follows:

1. To conduct fundamental investigations in the sanitary bacteriology and biology of shellfish and shellfish-bearing waters, with a view to the evaluation and improvement of presently accepted practices in the sanitary control of shellfish.

2. In collaboration with Federal Control Agencies, principally the Shellfish Sanitation Section, Milk and Food Program, Division of Engineering Services and Regional Office Personnel, to assist and guide the various states in their program of sanitary control of shellfish by rendering technical advice to the pertinent State Agencies.

3. In cooperation with Federal and State Agencies, to evaluate current commercial practices in harvesting, processing and marketing of shellfish and to develop and evaluate improvements in commercial practices. Many of these investigations are in the nature of collaborative studies undertaken with State Control Agencies. An example is the collaborative study to develop and evaluate bacteriological criteria for shellfish as received in the market areas.

4. To investigate laboratory methods for the examination of shellfish and shellfish waters for the purpose of evaluating current methods and developing new techniques. Again, work in this area is often in the nature of cooperative studies with state and federal control agencies. The current tentative standards of the bacteriological quality for Eastern Oysters at the market involves the use of a procedure which was developed during the course of an interstate cooperative study. The Shellfish Sanitation Laboratory was largely responsible for the development and preliminary evaluation of the method but cooperative investigations with states were necessary to determine the applicability of the test.

The activities of the laboratory have been so far within two geographical areas. The unit was first located at Woods Hole, Massachusetts where studies were made on the three commercial important species of shellfish in that area, the oyster, the hard clam and the soft clam.

In 1953 the Shellfish Sanitation Laboratory transferred activities from Woods Hole to Pensacola where investigations were conducted on the Eastern Oyster, the only commercially important species of shellfish in the area. After a stay of approximately five years the laboratory then transferred activities to its present location.

DESCRIPTION OF FACILITIES OF THE LABORATORY

An appropriation of $40,000.00 was made by the 1958 legislature for the construction of laboratory facilities. The laboratory buildings were moved from Vashon Island to the present location, the land being rented on a five-to-seven year contract from a private individual. The buildings required extensive renovation and reconstruction. The heating system, hot and cold water system, electrical wiring and related facilities are entirely new. The state also supplied the salt water system, which includes the intake, two electric pumps, a gas driven auxiliary pump, and the distribution system. In addition, the utilities, heat, gas, electricity, water and telephone service are furnished by the State Department of Health. The department also has employed a custodian to maintain the buildings and grounds. The Public Health Service has furnished the office and laboratory equipment and provides the scientific staff. The staff of the laboratory includes three scientific personnel, a biological aide and a stenographer.

THE SHELLFISH INDUSTRY IN THE PACIFIC NORTHWEST

There are a number of edible marine bivalves in the northwest coastal areas. Fitch* describes some 15 species in sufficient abundance in this area to warrant searching out for food purposes. Among these are three species of oysters, seven species of clams, three species of mussels and two species of scallops. Only seven of these species however are considered to be of importance commercially. They are:

1. The Pacific Oyster, Crassostrea gigas
2. The Native or Olympia Oyster, Ostrea lurida
3. The Native Little Neck Clam, Protothaca staminea
4. The Manila or Japanese Little Neck Clam Tapes Japonica
5. The Butter Clam, Saxidomas giganteus
6. The Razor Clam, Siliqua patula
7. The Kumo Moto Oyster, A southern variant of Crassostrea gigas

The commercial production of shellfish in the three West Coast States in 1957 is shown in Table 1. It can be seen that the Pacific Oyster is by far the most important species commercially. It must be pointed out that the figures shown in Table 1 are of the entire commercial production and a large proportion is heat packed as canned oysters or clams, stews and chowders. Estimates of the percentage entering the fresh stock trade are shown at the bottom of Table 1.

The nature of the Pacific Oyster is such that a fai y thorough cooking is required, although some receipes specify times and temperatures considerably below the point of destruction of bacteria. The native oyster is delicious raw and quite frequently served as an oyster cocktail. The Kumo Moto oyster is used similarly, although it is not uncommon to prepare both of these species in the form of fried oysters or oyster stews. Thus, because of the frequency of consumption of the native and Kumo Moto oysters in the raw or partially cooked state, the sanitary significance of the quality of the growing area is of greater concern than in the case of the Pacific Oyster.

In contrast to East Coast practices there is little if any sale of Pacific Oysters to the consumer in the form of shell stock. This is also true of the Native and the Kumo Moto oysters even though they are often consumed raw. Commerical traffic in shell stock takes place only to transport shell oysters to shucking houses at some distance from the harvesting area.

Contrary to East Coast practices is the fact that there are no West Coast species of clams which are consumed raw. The two little neck clams are used primarily for steamed clams or for chowders. The Butter Clam is excellent for chowders and in addition produces a delicious fried clam. The Razor Clam has poor keeping qualities and thus the shucked product is shipped in the trade. The mantle, foot and siphon only are used for food purposes. The gills and viscera are completely removed during the shucking operation.

*Common Marine Bivalves of California. John E. Fitch, California Fish Bulletin No. 90.

Table 1. COMMERCIAL PRODUCTION OF SHELLFISH IN THE WEST COAST

Species	Pounds of meat		
	Washington	Oregon	California
Pacific Oyster	9,855,000	429,000	1,330,000
Native Oyster	18,500	...	12,000
Other Oysters	5,600	...	17,000
Butter Clams	56,500	...	...
Little Neck Clams	220,200	...	...
Manila Clams	135,200	...	...
Razor Clams	318,000	27,000	...
Mixed Clams	...	24,000	4,000

References:

67th Annual Report (1957) Department of Fisheries, State of Washington

Fisheries Statistics of the United States, 1957, U.S. Dept. Interior Statistical Digest No. 44

These figures include commercial production for both fresh stock trade and for canning. Estimates of the relative volume for fresh stock trade are:

Pacific Oyster	1/ to 1/3
Native Oyster	All
Butter Clams	Practically all canned
Little Nock Clams	2/3
Manila Clam	3/4
Razor Clam	1/5 to 1/3

Conversion Factors: (Percent yield of shellstock as meat)

Pacific Oysters	
Puget Sound	10
Coastal	12
All hard clams (butter clams and little necks)	25
Razor Clams	42

Of the other species of clams, scallops and mussels mentioned by Fitch as being commercially used for food, the Pismo Clam and the Horse Clam or gaper probably would fall in the next order of consumption. At the present time both of these are primarily a sports product, being caught by private individuals for their own consumption. In California and Oregon, two species of mussels are also harvested by local residents for private use.

In consideration of the quality of production, methods of preparation of the product for consumption and extent of distribution, it would seem therefore that sanitation studies should be conducted on the following species:

1. The Pacific Oyster
2. The Native Oyster (and the Kumo Moto Oyster)
3. The Little Neck Clams
4. The Butter Clam

LABORATORY AND FIELD DATA COLLECTED AT PURDY SINCE NOVEMBER 1958

Upon completion of the laboratory facilities and trial runs on equipment, a program was established for the collection of information both on problems in methodology and of field conditions necessary for the formulation of a program of research activities. For example, information was needed on the quality of the sea water supply to determine the characteristics pertinent to the operation of experiments involving shellfish in the laboratory aquaria or tanks. In the nature of field investigations, trips were made to the producing and marketing areas in Washington, Oregon, California and British Columbia to review commercial practices in harvesting, handling and marketing of shellfish in order to learn of the problems in shellfish sanitation as well as to obtain information on the harvesting and processing practices.

THE LABORATORY SALT WATER SUPPLY

The salt water supplied to the laboratory is obtained from a point mid-stream just north of the Purdy-Wauna Bridge through a three inch intake some 300 feet long with pumps provided in the laboratory building. The salt water system was designed to provide continuous flow of water throughout the entire range of tides prevailing in this section. The tidal range as indicated by the U.S. Coast & Geodetic Survey Tide Tables has a maximum amplitude of three feet below to twelve feet above mean low tide.

A regularly scheduled program of sampling and examination of the laboratory sea water supply was started on December 22, 1958 and has continued to the present time. Samples of the sea water as pumped to the laboratory were collected twice daily Monday through Friday and once daily on Saturdays and Sundays. The samples were collected at the same time every day; 8 A.M. and 2 P.M. Thus, the samples were collected at different tidal conditions each day but the tidal stage was repeated at each two week tidal cycle. Coliform MPN was determined on all of the samples. The samples collected on Mondays and Tuesdays were submitted to the liquid confirmatory procedure for enumeration of E.C. gas positive organisms. All gas positive E.C. tubes were submitted to a classification of coliform type by the completed-IMVIC tests. In addition, membrane filter coliform counts were conducted on all of the samples collected from December 22, through April 10. The project therefore not only provided information on variation in coliform organisms in the sea water but also furnished a supply of samples on which to perform comparative tests to evaluate two of the recently developed methods for the bacteriological examination of sea water.

Table 2 is presented to illustrate the range in coliform MPN of the sea water.

The over-all range of coliform MPN in the samples examined between December 22, and May 30 was less than 1.8 to 9200, with monthly averages ranging from 11 to 30 as shown in table 2. The factors influencing the coliform density would appear to be a combination of rain-fall and tide. There appears to be an over-all correlation of increases in coliform density of the sea water with rain-fall during the previous two or three days and the coliform density was found to be highest in samples collected at the low tides.

During the course of the sampling program approximately 200 samples of sea water were submitted to tests designed to evaluate the liquid

Table 2. COLIFORM MPN, LABORATORY SEA WATER SUPPLY

Observed range	Dec. - Jan.	February	March	April	May
Minimum	2	1.8	1.8	1.8	1.8
Maximum	490	330	700	490	9200
MPN at 10 percentile*. . .	5.2	2.2	2.9	1.8	1.3
MPN at 50 percentile . . .	25	13	20	11	30
MPN at 90 percentile . . .	120	78	140	130	680

*Derived from plots on log probability paper.

confirmatory procedure for enumeration of E. coli type I and "fecal coliform organisms" in sea water. In more than three quarters of these samples the coliform types in the gas positive E.C. tubes were classified by the completed-IMVIC tests. These studies indicate the following:

1. The liquid confirmatory procedure is superior to the standard methods completed test for the isolation and enumeration of E. coli type I.

2. A positive E.C. confirmed test is quite reliable in defining E. coli type I. Classification of coliform types from 363 gas positive E.C. tubes has shown that in 332, or 92 percent, E. coli type I was present. The coliform organisms in the remaining 8 percent were predominantly aerogenes type I with a few E. coli type II.

The data on the comparison of the multitube dilution method with the MF membrane filter technique for the enumeration of coliform organisms in sea water have been compiled and submitted for statistical analysis to Dr. E.K. Harris, Chief, Statistical Services, Sanitary Engineering Center. The comparative study was designed to determine, in addition to the relationship in coliform densities determined by the two methods, certain details in methodology, such as the minimum number of replicate plates to produce a reliable count, and the influence of density of colonies, both coliform and non-coliform on the coliform count. A preliminary review of the data at this laboratory suggests the occurrence of considerable variation in the MPN/MF count relationships and therefore suggests that work should be continued on the evaluation of these two procedures. Design of further evaluations will be made after the statistical analysis has been completed by Dr. Harris.

The wide fluctuations in bacterial content of the laboratory sea water would introduce objectionable complexities if the water were to be used in certain of the proposed experiments. For example, in aquatium studies on the relative bacterial level of water and shellfish, a water of constant bacterial density would be needed for the production of reliable data. In such studies it would therefore be desirable to reduce the coliform density of the water to zero, provided the method of treatment had no adverse effect on other characteristics of the water as they related to its suitability for use in experiments. We therefore undertook a series of studies to evaluate the efficiency of ultraviolet ray irradiation in destroying coliform organisms in sea water. The equipment used at Pensacola was set up and samples of influent and effluent were taken fro bacteriological examination. Results obtained on 22 samples showed a range of coliform density in the influent of less than 1.8 to 460 with a median of 28. Of the 22 samples of effluent, all but one showed MPN's of less than 0.18. One showed an MPN of 2.0, equivalent to one of five 100 ml portions positive. Ultraviolet irradiation of sea water therefore appears to be a practical means of destruction of the coliform organisms. To test whether this method of treatment produced a water which had any influence on the feeding activity of oysters, two flats of shellfish were set up, one receiving ultraviolet ray treated water and the other the same water without treatment. Comparative activity counts on both flats indicated equal activity and thus that the treatment had no adverse effect.

BACTERIOLOGICAL TESTS ON SHELLFISH

Several tours were made through shellfish shucking houses in the Puget Sound Area, Willapa Harbor and other shellfish harvesting areas primarily to become acquainted with the commerical practices, with a view to the determination of the problems in the commercial harvesting, packing and marketing of shellfish. Samples of commercially handled shellfish were collected during some of these tours for bacteriological examination. The purpose of these tests was to determine the coliform and plate count levels prevailing in commercially handled shellfish and also to furnish a supply of samples on which to test the applicability of current bacteriological procedures to the West Coast species and conditions.

It was soon determined that modifications in the methods of preparation of sample for shellfish would be required. The commercial species of shellfish vary widely in size and physical characteristics and thus it was necessary to determine the optimum time of grinding of these species in commercial blendors. The extreme variation in size among the species required the development and evaluation of blendor jars both smaller and larger than those ordinarily used.

Work on optimum grinding time has been primarily on the Pacific Oyster, although a few

experiments have been conducted on the Native oyster. The results of the tests on the Pacific Oyster indicate that the optimum grinding time is between 75 and 150 seconds. Plate counts obtained on samples ground at 25 second intervals show a plateau within these two periods of grinding. Thus, the 90 seconds proposed for the Eastern species appears to be satisfactory. The extreme large size of this oyster however, has required the development and evaluation of a two quart blending assembly. This jar with a baffle designed to induce more complete circulation during grinding has been proven to be acceptable. The same blendor motor will accept containers as small as one-half pint in capacity and therefore can be used for blending the smaller species such as the native and Kumo Moto oysters.

A study to determine the variation in individual animals in plate count was undertaken. Single oysters were ground with an equal weight of dilution for 90 seconds in a small Waring Blendor assembly and 5 replicate plates in each of two dilutions were planted. From a preliminary review of the results it would appear that 10 to 12 oysters would be sufficient for a representative sample. We would however, prefer to await definite conclusions on these experiments until a statistical analysis is made of the results.

During the course of the visits to the shellfish producing and marketing areas we have collected 73 samples of shellfish taken at various stages of harvesting and marketing. A summary of the bacteriological results obtained on these samples is shown in Table 3. The bacteriological limits in the table conform to those recommended for Eastern species in the Public Health Service Manual of Recommended Practice.

The significance of these results of course, is not great because we have insufficient background information to allow interpretation of the results. The table is presented merely to show the degree of conformity with currently recommended criteria. It can be seen that in some cases the shellfish examined do not fall within the recommended levels for the East Coast. More intensive investigations over longer periods of time are therefore indicated.

Evaluation of the applicability of the liquid confirmatory test for "fecal coliform organisms" was conducted on all of these shellfish samples. The fidelity of the E. C. gas positive test in demonstrating the presence of E. coli type I was close to that which we observed in similar studies on oysters in Pensacola. In the Purdy studies, 90 percent of the E. C. gas positive tubes were positive for E. coli type I. This compares to 81 percent obtained on oysters in the Gulf Coast. 18 E. C. gas positive tubes from which E. coli type I was not isolated aerogenes type I was most often found. Twelve of the 18 tubes were found to contain this coliform

Table 3. BACTERIOLOGICAL SURVEY OF COMMERCIALLY HANDLED SHELLFISH

Number of samples	Pacific oysters			Olympia oysters		Clams	
	Shell	Packed	Market	Shucked	Market	Plant	Market
Coliform MPN/100 ml							
230 or less	4	2	6	1	2	0	1
More than 230 . . .	18	18	11	4	3	1	2
E. C. Gas Positive MPN/100 ml							
78 or less	16	14	14	4	5	0	2
79 to 230.	4	4	0	1	0	1	0
More than 230 . . .	2	2	3	0	0	0	1
Plate Count per ml							
50, 000 or less . . .	22	18	16	4	4	1	3
51, 000 to 100, 000 .	0	1	0	1	1	0	0
More than 100, 000.	0	0	1	0	0	0	0

type. E. coli type II, Aerogenes type II, Intermediate type II and an atypical IMVIC type were isolated from the other 6 tubes.

The behavior of the liquid confirmatory procedure was therefore quite similar to that observed in the evaluation of the procedure during the 1958 East Coast market study. Thus, at least similar coliform types are retained by the test in both areas. The sanitary significance of E. coli type I, or in fact, any coliform type in shellfish is at the present time not completely clear. There is a need for studies in shellfish growing areas that would associate the findings of a comprehensive sanitary survey with the presence or absence and the relative numbers of E. coli type I "fecal coliform organisms" and other extraneous forms.

ENUMERATION OF SALMONELLAE IN SEA WATER AND SHELLFISH

Preliminary investigations on methods and procedures to be used in studies on the survival of enteric organisms in shellfish have consisted of experiments concerned with evaluating the methods for enumerating salmonella using experiences in Pensacola as a guide.

The first experiment was to determine whether the methods would satisfactorily enumerate salmonellae in shellfish freshly contaminated with pure cultures. The oysters used in these tests were stored in the refrigerator for varying lengths of time in an effort to detect the effect of changes occurring during storage. The oysters were first ground in a Waring Blendor and suspensions of Salmonella schottmuelleri then added. Plate counts on the suspensions were conducted to determine the number of organisms added. The sample was pre-enriched in lactose broth, transferred to two selective enrichment broths, and confirmed on two differential plate agars. The results indicated a quantitative enumeration was obtained by all the methods employed with the exception that one of the differential agars, brilliant green agar, failed to isolate salmonellae effectively due to overgrowth.

We, therefore continued with experiments to determine whether storage of salmonellae with oysters would produce any changes which would influence the recovery. Contaminated oysters were stored at 17°C and 23°C to accelerate changes in the oysters and to determine if salmonella would multiply at those temperatures. This oxporiment demonstrated multiplication of salmonella but enumeration was erratic. Skips, apparently associated with the sample/medium ratio occurred in various dilutions.

In an attempt to rectify this difficulty, tests were made using more medium per unit weight of oyster meats, but those were inconclusive since "skips" occurred in the controls of only one of the twelve samples tested.

Further evaluation studies, are necessary to develop procedures satisfactory for enumerating salmonellae. This has been a major problem in studies on the comparative survival of enteric organisms in sea water and shellfish.

Appendix C

PROPOSED PROGRAM OF RESEARCH ACTIVITIES

Prepared by

The Staff of the Shellfish Sanitation Laboratory

PROJECT I

EVALUATION OF THE SANITARY SIGNIFICANCE OF "FECAL COLIFORM ORGANISMS" IN SHELLFISH AND SHELLFISH WATERS

PROBLEM AND OBJECTIVES

The 1958 Shellfish Sanitation Workshop adopted a new two-year interim bacteriological standard for market oysters based on E. C. gas positive coliform organisms and Plate Count. The revision of the standards developed from a review of the results of co-operative studies of the bacterial changes in shellfish during shucking, packing, and marketing which demonstrated that the entire coliform group of organisms was not a meaningful indicator of pollution or fecal contamination in shucked oysters as marketed. The E. C. gas positive, or "fecal coliform organisms" appeared to be a better index of pollution and the plate count a good index of poor plant design or operation, improper refrigeration, or other types of mishandling that would result in general bacterial multiplication.

It was quickly pointed out at the Workshop that there was insufficient evidence to establish the true fecal significance of either E. coli type I or the E. C. gas positive group of coliform organisms, and the Public Health Service was requested to prepare a uniform procedure for an interstate co-operative study in shellfish growing areas to determine the degree of association of these organisms with observed fecal pollution of human or animal origin. A proposed outline of study has been prepared by Eugene T. Jensen and C. B. Kelly and has been submitted to the States for review and comment. A copy of this outline is attached.

The study involves comprehensive sanitary surveys of shellfish growing areas, both approved and restricted, with a view to the correlation of numbers of coliform organisms, "fecal coliform organisms", and E. coli type I found in the shellfish growing area with the findings of a sanitary reconnaissance which would determine the sources of pollution and quantities of index organisms emanating from such discharges. A hydrographic survey of the marine area and tributaries would determine the flow time of pollution to the growing area and the extent of dilution with fresh and salt water.

Four states on the Eastern Seaboard and one on the Gulf Coast have indicated their interest to participate in this co-operative study and the states of Oregon and California propose to include tests for "fecal coliform organisms" in their routine sanitary surveys. The Department of National Health and Welfare of Canada is also participating in the co-operative study. The Washington State Department of Health is co-operating with the Shellfish Sanitation Laboratory in sanitary surveys of shellfish growing areas in the vicinity of this laboratory.

PROCEDURE

In our participation in this study, we have first selected as a test area, Burley Lagoon, the watershed and estuary immediately adjacent to the Shellfish Sanitation Laboratory. Thus, the data collected in this project will have the additional value of furnishing information on the bacteriological quality of the sea water supplied to the laboratory.

This study is in progress. The Washington State Department of Health is conducting a comprehensive sanitary reconnaissance of the watershed. Mr. John Girard and Mr. Max Hayes of the Division of Engineering and Sanitation are inspecting every potential source of pollution whether it be of human or domestic animal origin and in addition will survey the stream for potential contribution of coliform organisms from marshy areas or other sources.

The Shellfish Sanitation Laboratory has conducted the first bacteriological survey of the area. During the period March 30 to April 24, seven sampling tours were conducted. Ten stations were established, four on the contributing streams, five in Burley Lagoon (One of these is the sea water supply for the laboratory), and one in Henderson Bay, the outer estuary. Samples were examined for density of coliform and "fecal coliform" organisms and approximately one-half were submitted to the completed IMViC procedure for classification of coliform organisms isolated from E. C. gas positive tubes.

The bacteriological survey showed the presence of coliform organisms in moderately high numbers in the upper section of Burley Lagoon. A large proportion of these were found to be E. C. gas positive. The sanitary reconnaissance should indicate the source of these coliform organisms, but there is a likelihood that they in part come from fecal discharges of human or animal origin.

It would be desirable to investigate an area of similar hydrography but with less contributing human and domestic animal population. Preliminary surveys suggest that the northern section of Case Inlet, locally called North Bay might be a suitable area.

The suggested procedure recommends a frequency of sampling. It recommends that intensive sampling should be carried out for at least one week per quarter during the shellfish harvesting season with an additional preseason sampling when necessitated. The procedure also recommends the collection of at least two sets of water samples per station per day during each sampling period.

We would propose to conduct sampling trips on each of these two bays during two weeks of each quarter from the period March 30, 1959 to March 30, 1960. Two sampling tours would be conducted during each week, resulting in the collection of eight samples per station per quarter. Coliform MPN and E. C. gas positive MPN would be determined on all samples. Between 25 and 50% of the samples would also be submitted to a completed test on the E. C. gas positive tubes, with classification of the coliforms isolated by the IMViC reactions.

A report for each area will be prepared for distribution and discussion at the 1960 Shellfish Sanitation Workshop. We propose to follow the format as outlined in the suggested procedure for the co-operative study.

PROJECT II

DEVELOPMENT AND EVALUATION OF METHODS FOR THE EXAMINATION OF SEA WATER AND SHELLFISH

PROBLEMS AND OBJECTIVES

The development of methods for the examination of shellfish and shellfish waters was based primarily on experiences on the Eastern and Gulf Coasts. Routine bacteriological methods, such as the tests for coliform organisms in sea water have been applied sufficiently on the West Coast to indicate the reliability of these methods in this area. However, certain tests of more recent development, like the membrane filter technic and the liquid confirmatory procedure for the enumeration of "fecal coliform organisms" have not been widely used and their reliability when applied to west coast conditions is not known.

The fact that the species of shellfish on the Pacific Coast are entirely different than those of theAtlantic and Gulf Coasts indicate the need for modification of methods of examination to render them applicable to the Pacific Coast species. The shellfish on this coast vary widely in size and other physical characteristics to the extent that even the methods of grinding will require modification.

Before undertaking large scale routine bacteriological testing in this area, it therefore became necessary to initiate investigations to determine the applicability of currently accepted methods to the West Coast conditions.

PROCEDURE

Methods for Sea Water

Since December 1958, a program of sampling and examination of the laboratory sea water supply has been conducted. Although it was initiated primarily to determine the suitability of the water for certain proposed shellfish experiments, it has provided a series of water samples valuable for the evaluation of methods for the bacteriological examination of sea water.

As indicated in the statement of accomplishments, the sampling program has already furnished data for an evaluation of the MF technique in estimating coliform densities. Comparative MF counts and coliform MPN's from 184 samples collected from December 22, 1958 through April 10, 1959 have been submitted for statistical analysis to Dr. E.K. Harris, Chief, Statistical Services, Sanitary Engineering Center.

Preliminary review of the MF data reveals periodic variations in the MF count/MPN coliform relationships. Since the MF coliform count depends on the production of recognizable, typical colonies on the membrane, these variations suggest a study to determine the frequency of occurrence of coliform organisms producing "atypical" colonies and non-coliform organisms producing "typical colonies".

An evaluation of the E.C. test as applied to sea water was initiated on December 22, 1958 and has continued to the present time. One hundred and twenty samples of sea water have been submitted to comparative tests to determine the reliability of the method in defining "fecal coliform organisms".

However, the MF and EC evaluations have encompassed only the winter and spring periods and therefore have not revealed possible variations during the other seasons.

The use of enterococci as a supplementary indicator organism has been under consideration for a number of years. Unfortunately, methodology has not been refined to the point of the production of a simple and reliable method

of enumeration. In the past two years, there has been renewed interest in the development of methods for the enumeration of these organisms in water. Recently there were developed two new techniques, one a multitube dilution method and the other an M. F. procedure. The applicability of these to sea waters on the west coast should be investigated with a view to including one or the other in the sanitary survey project.

We therefore propose the following program of investigation for the remainder of the calendar year:

1. A continuation of the program to determine the MF count/MPN coliform relationships throughout the remaining cycle of seasonal variations.

2. A continuation of the program to determine the validity of the E. C. liquid confirmatory method.

3. Initiation of an investigation to determine the fidelity of the MF technique in demonstrating the presence of coliform organisms. In the beginning we propose to classify "typical" or "atypical" coliform organisms from membranes prepared by the standard methods procedure which uses M. Endo medium without pre-enrichment. As other media and techniques are developed these will also be investigated.

4. Initiate an evaluation of the two recently developed methods for the enumeration of enterococci, using as source material samples of sea water collected during the course of sanitary surveys and of the routine program of sampling the laboratory sea water supply. The evaluation would be conducted in a manner similar to that followed in the evaluation of the M. F. procedure for coliform organisms.

Shellfish Tests

Preliminary investigations have established the necessity for modification of the methods recommended for shellfish. It has already been determined that it will be necessary to modify the methods of preparation of the sample to adapt the procedures to the Northwest species. The grinders currently in use are not adaptable. For example, the large size of the Pacific Oyster and certain of the clams requires a larger vessel than that ordinarily used to accomodate a representative number of animals. On the other hand, the extreme small size of the native oyster has required the development of a container much smaller in size than customarily used. Although we have sufficient data to indicate the optimum grinding time of the Pacific Oyster, there still remains the establishment of optimum grinding time of the other commercially important species.

It has been mentioned that we have a quantity of data on the evaluation of liquid confirmatory procedure for "fecal coliform organisms" in shellfish, but since the work has been done only during the months of December through February, the influence of seasonal variation on the fidelity of the test has not been established.

We would therefore propose the following action in investigation of methods for the examination of shellfish:

1. Determination of the optimum grinding time of other commercially important species of shellfish. The two little neck clams, the butter clam, and the native oyster should be studied.

2. A continuation of the evaluation of the liquid confirmatory procedure for "fecal coliform organisms" to detect possible seasonal variations. In project V, we propose a regular schedule of sampling in commercial shellfish establishments. These samples should furnish an ample supply of material on which to conduct these comparative tests.

PROJECT III

ACCUMULATION AND ELIMINATION OF BACTERIA BY SHELLFISH

PROBLEM AND OBJECTIVES

Assessment of the sanitary quality of shellfish as harvested is based on a sanitary survey of the growing area which determines whether the area is influenced by fecal discharges that may contain pathogenic micro-organisms. Thus, the assessment of the sanitary quality of a growing area is based primarily on an estimation of the quality of the over-lying water. It is known that shellfish in their normal feeding processes accumulate bacteria and therefore their relative bacteriological level is higher than that of the over-lying water. In order to assess the suitability of a growing area it therefore becomes necessary to know the rate and extent of accumulation of bacteria by shellfish.

It is also necessary to know the rate and extent of elimination of bacteria by shellfish when transplanted from a polluted to a clean environment. With the increases in population on marine watersheds, the problem of maintaining the sanitary quality of shellfish growing areas is growing increasingly difficult.

Many species respond well to a process of purification involving transplanting from a polluted to a clean area. Others, because of peculiarities in habitat or of susceptibility to destruction by predators must be provided with captive environment.

The changes taking place in a natural estuary and particularly in the intertidal zone are extremely complex. Variations in tidal amplitude, time of tide, fresh water run off, air temperature, and many other factors produce an aquatic environment which is continually changing, bacterially as well as in other respects. Among the factors in this complex system that might influence the water-shellfish relationships are

1. Variation in length of time the shellfish are under water.
2. Fluctuations in bacterial content of water with tidal changes.
3. Changes in bacterial population of oysters on the beds during exposure to the air.
4. The effect of fresh water dilution on the pumping rate of shellfish.
5. The influence of turbidity and other particulate matter on the pumping rate.
6. Seasonal variations in pumping activity.

The extreme complexity of the natural conditions would make it necessary to study some of the factors listed above separately under controlled conditions to obtain some idea of the relationships between the two or three factors under investigation.

PROCEDURE

We would therefore propose that a series of experiments be conducted under controlled laboratory conditions throughout a calendar year to encompass seasonal variations. Simultaneously, limited studies should be conducted in the adjacent estuary to correlate laboratory and field findings. The objectives of these experiments would be

1. Under controlled laboratory conditions, to determine the rate of accumulation and elimination of bacteria by the commercially important species of shellfish.
2. By parallel studies in a natural estuary to determine the relationship in rate of accumulation and elimination between laboratory and natural conditions.

It is highly improbable that we will be able to conduct intensive investigations on all of the

commercially important species. As judged by quantity of production, the Pacific Oyster is by far the most important and should receive the most attention. However, the Olympia Oyster, because it is frequently consumed raw, assumes significance beyond its importance production-wise. This species should also be investigated.

It would be possible to study one of the two little neck clams and the butter clam simultaneously. We have little scientific information to justify whether either of the little neck clams is representative of the two species. The relative production appears to vary from year to year. We would propose to investigate their similarily first, then select one or both for study with the butter clam.

The shellfish would be placed in tanks under conditions resembling the natural habitat. The oysters would be placed directly on the bottom of the laboratory tanks. It might be necessary to provide a bed of clean coarse sand or gravel for the clams, particularly the butter clam. The shellfish would be supplied with sea water of controlled bacterial level, preferably at two levels simultaneously and by examining the water and shellfish periodically the relative bacteriological content would be determined.

Because of the fluctuations in coliform density of the sea water it would be necessary to reduce the coliform content to zero prior to the addition of polluting cultures. Treatment of sea water by irradiation with ultraviolet light has been established as an effective means of accomplishing this. We would however, set up flats receiving purified water to serve as control on the efficiency of the purification system and on the behavior of the test flats.

At the end of the experiment, the polluted shellfish remaining would be supplied coliform free water and, by sampling after two or three periods of exposure to this water, the rate and extent of purification would be determined.

Simultaneously, a tray of these shellfish would be placed on the bottom of the bay adjacent to the water intake. Periodic examination of the raw water and of the shellfish should furnish information on the rate of accumulation under these more natural conditions and should allow comparison with the results obtained under captive conditions.

A similar comparison in purification could be conducted by transplanting a stock of the laboratory polluted animals to a tray kept in the bay near the intake. Examination of water and shellfish would be conducted periodically to determine the time required for the shellfish to reach equilibrium with the water.

We should provide sufficient quantities of water to the shellfish to attain equal activity throughout the tank. Preliminary studies should be conducted to determine the water flows required. Experimental flats should be set up containing quantities of shellfish needed for the experiment and the sufficiency of the water should be determined by bacteriological or physical tests on the water and shellfish at each end of the tank.

Index tests that may be used are

1. Bacterial: Coliform preferably
2. Measurement of quantities of feces and pseudo-feces
3. Dissolved oxygen of the water

When the water requirements have been determined under the most adverse conditions, the design of experiment can be completed.

There is a definite seasonal variation in the activity of molluscs. The Pensacola studies showed two periods of high activity and two periods of low activity, both of the latter associated with temperatures, one high and the other low. The influence of salinity should also be considered.

The experiments should be scheduled to encompass seasonal variations encountered in the Pacific Northwest. Replications should therefore be made at the following periods of the year:

1. December through February, low water temperatures; reduced salinity
2. April through May - moderate temperature; higher salinity
3. July through August - warm temperature; high salinity
4. Mid-September through October warm temperature - reduced salinity

At least duplicate experiments should be conducted during each of the seasons.

Relationships in a Natural Estuary

The experiments described above should provide water-shellfish relationships at two levels of pollution, seasonal variations, and species differences. The simultaneous studies in the natural estuary should show the relationships between laboratory and field conditions. It would seem that such basic relationships are needed to assist in the interpretation of findings under the more complex conditions in a natural environment.

It is recognized that the conditions of the laboratory experiments are only those of captive condition in an environment in which there is little change, and thus investigations in the field are necessary.

The approach to the field studies should be to first determine areas in which some of the factors such as water quality are typical, but fairly constant, establish stations at various tide levels, and by sampling throughout the entire tidal cycle and at different climatic conditions, determine the changes that take place in the water and shellfish. Such studies should be carried out in several shellfish producing areas in an effort to cover geographical or hydrographic differences.

PROJECT IV

SURVIVAL OF ENTERIC ORGANISMS IN SEA WATER AND SHELLFISH

A. SURVIVAL OF ENTERIC ORGANISMS IN SEA WATER AND SHELLFISH

Problem and Objectives

In the processes of feeding and respiration shellfish filter water. Bacteria in addition to plankton and suspended organic matter are accumulated on the body and in the shell liquor of the shellfish. Because of this biological activity, shellfish in waters subject to fecal contamination can accumulate enteric pathogens and become a factor in transmitting enteric disease. In view of this the production of safe shellfish has depended on the assurance that they are harvested from waters remote from sources of dangerous fecal pollution.

Since the presence of dangerous fecal pollution cannot be demonstrated by the isolation of enteric pathogens, resort has been made to indicator organisms associated with fecal pollution. These include the coliform group of organisms. The use of an index organism makes a knowledge of the relative survival time of indicator and pathogenic organisms in sea water necessary. The evidence that factors present in sea water associated with salinity and season may affect the survival of enteric organisms indicates the need for study under these different conditions.

Therefore, the purpose of this study is to determine the comparative survival time of coliform organisms and enteric pathogens in sea waters of different salinity at different seasons of the year.

Procedure

The proposed plan of study is as follows:

1. The survival of enteric organisms would be studied in high and low salinity waters at each of the four seasons encompassed by a calendar year.

2. This study would be made on natural sea waters, inoculated with the laboratory strains of coliform and salmonella organisms used in previous studies. The coliform organisms would consist of three coliform types, E. coli, intermediate, and aerogenes, and the salmonella of three strains from different serological groups. The water would be stored in 5 gallon bottles partially immersed in a tank of flowing sea water to produce temperatures similar to that in the natural estuary immediately adjacent to the laboratory.

3. The concentration of the organisms added to the sea water would be approximately 10,000 coliform and 1,000 salmonella per 100 ml.

4. The end point of each experiment would be the failure to isolate viable salmonella from 100 ml portions in two consecutive samples of test water.

5. Samples would be taken at regular intervals, the same sampling schedule to be used in all experiments.

6. Quantitative methods would be used for enumerating the test organisms.

Certain preliminary investigations are indicated. One of these is to determine the source of high and low salinity waters and the other an evaluation of the methods to be used for enumerating salmonella.

Since laboratory strains of indicator organisms in addition to the pathogens are to be used for this study the test waters should not contain large numbers of coliform organisms. It would

appear on the basis of results of bacteriological examinations conducted that the water supplied to the laboratory would be a suitable source of high salinity water, particularly if taken at high tide. However, preliminary surveys should be made to determine the source of a suitable low salinity water (10-15 ppt) whose native coliform content is similar to that of the high salinity water.

Methods for enumeration of salmonella in sea water should be evaluated. Some studies in Pensacola indicate that a more constant and higher recovery of salmonella is obtained by pre-enrichment in a non-selective medium prior to enrichment in a selective, but more inhibitory medium such as tetrathionate broth. Studies should be conducted to determine the efficiency of this method on freshly inoculated and stored water. These are necessary since it has been observed that the efficiency of the method for enumerating salmonella may be affected by some unknown factor associated with storage.

B. SURVIVAL OF ENTERIC ORGANIMS IN SHELLFISH

Problem and Objectives

Though there are data that show that enteric pathogens can survive for the entire market life of eastern oysters and soft clams little quantitative information is available for other species of shellfish.

Since a criterion of the value of an indicator organism is its relative survival and behavior as compared to that of enteric pathogens, multiplication of coliform organisms in shellfish has raised the questions:

1. Whether enteric pathogens can survive and multiply in shellfish in a similar manner.

2. Whether the coliform group of organisms is a satisfactory index of the sanitary quality of shellfish.

The results of recent studies have indicated that "fecal coliform organisms" as determined by the liquid confirmatory procedure is a better index of the sanitary quality of Eastern oysters at the market than the coliform group of organisms.

It would be desirable therefore to determine the relative behavior of coliform, "fecal coliform" and salmonella organisms in shellfish during conditions simulating commercial handling. This information will be of assistance in assessing the value of the two indicator organisms as a criterian of the sanitary quality of market shellfish.

Since a variety of commercial species of shellfish exist in the Northwest, the study would be limited to the most valuable species. It is proposed to study the survival of enteric organisms in Pacific and Olympia oysters, one of the species of little neck clams, and possibly the butter clam, with emphasis on the oysters.

Procedure

Although no definite procedure of study can be prepared until details of methodology are investigated, the following principles of procedure are proposed:

1. Quantitative methods of enumeration would be used to trace the relative behavior of index organisms and enteric pathogens in shellfish.

2. Shellfish would be stored at two temperatures. One would be the refrigerated temperature usually used in commercial practice; the other at which salmonella and indicator organisms may multiply.

3. Storage time would be at least as long as shellfish might be in transit between harvesting and consumption and for an extended period thereafter while they are still in marketable condition.

4. Samples would be taken at regular intervals, constant in all experiments. The intervals would be governed by storage temperature and the storage life of the shellfish.

5. Laboratory strains of salmonella and coliform organisms used in previous studies would be used. These would consist of a mixture of three coliform types, one of which is a "fecal coliform" type, and three salmonella strains, of different erological group. The shellfish used for this study would contain approximately 10, 000 coliform and 1, 000 salmonella organisms per 100 ml.

6. Since shellfish are harvested on a year around basis in this area, replicate experiments for each species should be conducted at each of the four calendar seasons.

The results of studies at Pensacola and of some preliminary experiments in Purdy indicate that a number of problems exist which must be investigated before the actual experiments are started.

The problems that need investigation follow:

1. Evaluation of methods for enumerating salmonella in shellfish.

2. The determination of the extent of multiplication of salmonella in shellfish at or near the highest storage temperature encountered in commercial handling and the temperature at which salmonella and indicator organisms will multiply in shellfish. This information is needed to establish the higher of the two storage temperatures.

Past experience in other areas had indicated that commercial conditions of storage of shucked shellfish can be simulated in the laboratory. The relative behavior of indicator organisms and specific pathogens as traced under laboratory conditions can therefore be translated directly into commercial practices.

It has also been our experience that it has not been possible to produce storage conditions in the laboratory which would result in behaviors in shell stock similar to those observed in the field. Invariably the degree of bacterial multiplication is much lower in the laboratory than in good commercial storage.

It would therefore appear that studies on the relative multiplication of index and pathogenic organisms should be carried out in shucked shellfish and that studies on the extent of multiplication of index organisms in shell stock be done in project V which is designed to determine the bacterial changes in commercial practice.

PROJECT V

INVESTIGATIONS OF COMMERCIAL PRACTICES OF HARVESTING, PACKING AND MARKETING OF SHELLFISH

PROBLEM AND OBJECTIVES

Many of the commercial harvesting practices in the Pacific Northwest are unique to this territory. They are necessitated partly by the natural habits of the species and by extreme variation in tides. Furthermore, the extreme tide levels result in unusual conditions of time of exposure and variation in water quality presented to the shellfish. This is true of all of the commercially important species with the exception of the native oyster. Harvesting methods vary from area to area. In Willapa Harbor oysters are harvested by dredging at high tides. In the lower Puget Sound, dry harvesting is practiced at low tides.

To maintain stocks of shellfish during periods of low amplitude of tide and during adverse weather conditions, it is general practice to harvest in excess of demand and to hold the oysters either by wet storage in sink floats or by dry storage on barges or in the shellfish houses. There is no information on the influence of these conditions on the sanitary quality of the shellfish.

Furthermore, there is needed additional information on the bacterial changes taking place during commercial practices of shucking, packing, and transportation to the market. This information is needed for assessing the sanitary conditions of harvesting and packing methods and for the assessment of the sanitary quality of the product at the market level.

PROCEDURE

We therefore propose to establish a regular sampling program in representative shellfish processing and harvesting areas, to study the changes taking place in commercial practices of harvesting, processing, and marketing of shellfish. Studies should be conducted on traced lots of shellfish collected at strategic points in commercial packing and marketing.

Sink float operations should be studied. Information is needed on the water-shellfish relationships in sink floats to determine the influence of high density shellfish population on their bacterial content. Bacterial changes during dry storage should be investigated. Time-temperature relationships of the bacterial content of shellfish during commercial dry storage should be determined.

Sampling in each area would be conducted on a quarterly basis throughout at least one calendar year in order to encompass seasonal variations. Records will be kept of observable factors which might effect the quality of shellfish e.g., general sanitary conditions in plants and markets, air and shellfish temperatures, storage conditions of the shellfish prior to shucking, holding interval of shucked shellfish prior to packing, storage conditions of the packed product, storage conditions during transit and at the market. General sanitary conditions in the plants and markets should be based on ratings by state control agencies.

Samples would be tested for coliform organisms, plate count and "fecal coliform organisms", the latter by the E. C. liquid confirmatory procedure. The tentative market standards for Eastern oysters are based on the results of the E. C. liquid conformatory test and the plate count and therefore these tests are indicated on Western species to determine the degree of compliance with the Eastern market standards. The coliform MPN is proposed to determine the degree of conformity with current recommended bacteriological levels at the point of harvesting and packing as contained in the manuals of recommended practice. The E. C. liquid confirmatory procedure should be evaluated in this territory and we would therefore propose the completed-IMVIC test on a sufficient number of samples to determine the behavior of the test on Northwest species of shellfish.

Appendix D

SUGGESTED PROCEDURE FOR EVALUATING THE SANITARY SIGNIFICANCE OF FECAL COLIFORM ORGANISMS IN SHELLFISH AND IN SHELLFISH WATERS

E. T. Jensen and C. B. Kelly

INTRODUCTION

The need for a bacteriological index of the sanitary quality of market shellfish has been apparent for many years. Thus, since 1950 there have been several cooperative investigations of the changes in bacteriological quality of shellfish during shucking, packing, and marketing. These studies have resulted in changes in the recommended procedures for shucking, packing, and refrigeration of shellfish. They have also lead to the adoption of a sequence of interim bacteriological standards for shucked Eastern oysters as marketed.

Control agency experience with the 1956 interim bacteriological standard for market oysters was reviewed at the 1958 Shellfish Sanitation Workshop. It was unanimously agreed that the entire coliform group of organisms were not meaningful indicators of pollution in shucked oysters as marketed. The Workshop, therefore, adopted a new two-year interim standard based on E. C. gas-positive coliform organisms and the Plate Count. The Workshop agreed that the sanitary significance of such a standard could not properly be established without reliable information on the relationship of the E. C. gas-positive coliform organisms in shellfish to observed sewage pollution of growing areas, and recommended that studies be undertaken of this relationship. The Public Health Service was requested to develop a uniform procedure for use by the States in undertaking these investigations. It was agreed that the preliminary results of these studies would be discussed at the 1960 Shellfish Sanitation Workshop.

SCOPE

Nationally, the studies should include all commercial shellfish species in all important producing areas; however, it is not anticipated that a State would undertake more than one such study. The PHS Shellfish Sanitation Laboratory will probably participate in the West Coast study.

REPORTING OF RESULTS

Preliminary reports to be presented for discussion at the 1960 Shellfish Sanitation Workshop should preferably be of uniform format and should include all facts and data necessary for comparative review. A mere summary of bacteriological results without supporting sanitary survey and hydrographic data will not be helpful in determining the sanitary significance of fecal coliforms in shellfish and shellfish growing waters.

SELECTION OF STUDY AREAS

Each study should encompass two small growing areas of comparable size and hydrography, but having significantly different levels of pollution, i.e., one clean, one polluted. The selection of relatively small areas wili facilitate the intensive study which will be necessary, and will minimize the number of variables to be considered in evaluating the data.

SANITARY RECONNAISSANCE

The Purpose of the sanitary reconnaissance is to locate, identify and evaluate every potential source of coliforms and fecal coliforms in the estuary and its tributary watershed. The sanitary reconnaissance will require a quantitative evaluation of every such pollution source including municipal sewerage systems, individual sewage disposal systems, boat traffic, recreational use, farms, large population of wild animals or fowl, industrial wastes, and extensive swamps or mud flats. Each pollution source must be plotted in terms of distance and estimated time of flow from the growing area under investigation.

Special consideration must be given to the adequacy of sewage treatment works. Such units should be described in detail and provisions should be made for determining the operating effectiveness of the unit immediately prior to and during bacteriological sampling.

The sanitary reconnaissance must be continuous throughout each bacteriological sampling period to establish that there has been no significant change in pollution sources in the estuary or its tributaries.

GEOGRAPHY

Any geographic elements having a bearing on the bacteriological quality of the estuary and its tributary rivers should be investigated and described. Department of Agriculture aerial photographs should be used where possible.

HYDROGRAPHIC SURVEY

The objective of the hydrographic survey is to predict what proportion of the observed pollution reaches the growing area, and to aid in the establishment of the bacteriological sampling stations. The hydrographic aspects of each study should preferably be planned and carried out under the direction of an experienced hydrographer. The survey should be continuous during each period of bacteriological sampling.

The hydrographic survey should, wherever possible, determine the flow times from each point of pollution to the growing area. The possibility of stratification in the growing area should be considered, and checked during each bacteriological survey period. The use of recording tide and river stage gauges should be considered wherever possible so as to obtain the best possible data. (Note: The hydrographer will be concerned with specific conditions in the area at the time of bacteriological sampling rather than with average conditions over a longer period of time).

BACTERIOLOGICAL SAMPLING

In establishing the water sampling frequency it must be recalled that the purpose of these studies is to develop comparative bacteriological data on clean and polluted areas which can be used as scientific justification for bacteriological standards for shellfish and shellfish growing waters.

Ideally, bacteriological sampling should be carried on at a relatively high frequency over at least a two year period to give adequate consideration to seasonal and hydrographic variations. It is apparent that such a sampling regimen would ordinarily be impractical because of personnel and laboratory limitations. Considering the need for coordinating all bacteriological sampling with sanitary reconnaissance and hydrographic information a program of repeated, short-term high frequency sampling is recommended. Sampling should also be conducted whenever significant changes in hydrographic or pollutional conditions occur and for a sufficient time thereafter to allow the correlation of bacterial findings with the hydrographic or other data.

The necessary frequency of sampling depends, of course, upon the areas under consideration. However, intensive sampling should be carried on for at least one week per quarter during the shellfish harvesting season with an additional pre-season series where necessary to establish the bacteriological quality of the water prior to the harvesting season. At least two sets of water samples should be collected per station per day during each sampling period. The depth of sampling should be based on the recommendation of the hydrographer. Both top and bottom samples may be required in the event of stratification. Each sample should be accompanied by the related salinity and temperature information. Salinities should be expressed in parts per thousand, and may be determined from the water temperature and specific gravity using

Knudsen's hydrographic tables for conversion to salinity.

Sampling stations should be so selected that the results obtained will be representative of the bacteriological quality of the growing areas during both flood and ebb tides.

While most of the laboratory work will be concentrated on water some shellfish should also be examined. It is therefore recommended that shellfish samples be collected at approximately 10% of the water sample stations. The sampling frequency for shellfish at these selected key stations might be approximately one-half that of the water sampling frequency. Samples of more than one species of shellfish should be collected for examination whenever possible. The bacteriological examination of bottom sediments is not recommended because of the difficulties in standardizing this procedure.

Sewage treatment plant effluent should be sampled on the same frequency as the growing area water. Effluent of critical sewage treatment plants should be sampled on the same days that the other water and shellfish samples are collected. In some instances effluent sampling a day or two in advance of area sampling may be necessary. Composite samples of sewage treatment plant effluents should be obtained wherever possible.

SAMPLE HANDLING

APHA Standard Methods should be followed.

LABORATORY METHODS

Application of the E. C. presumptive test has been justified for use with the examination of shellfish at the market level; however, information is not available which warrants a similar assumption for the growing area study. Therefore, the complete IMViC test should be conducted on all gas positive E. C. tubes on alternate samples to evaluate the E. C. presumptive test. The completed IMViC test may be omitted after the first year if it is found that consistently reliable results are obtained.

A recommended bacteriological procedure for coliform MPN, fecal coliforms, and plate counts will be found in Appendix I.

PREPARATION OF COMPREHENSIVE REPORT

A preliminary report for each area should be prepared for distribution and discussion at the 1960 Shellfish Sanitation Workshop. The report should describe the area in sufficient detail to enable persons attending the Workshop to understand and evaluate the comparative studies.

For ease in comparison of data the following format is suggested:

Part 1. Narrative description of the area including photographs where available. Department of Agriculture aerial photographs may be used.

Part 2. Area map showing the entire watershed with points of significant pollution indicated. The Geological Survey maps are well suited for this purpose.

Part 3. Chart of area being investigated with bacteriological sampling points indicated. Coast and Geodetic Survey charts may be well suited for this purpose.

Part 4. Sanitary Reconnaissance. This section should include detailed information on each source of coliform and fecal coliform organisms. Any sewage treatment plants in the watershed should be described in detail. Photographs should be used wherever possible.

Part 5. Hydrography. The section should give information on the discharge and flow time of tributary rivers, wind and tidal currents and in the areas under study, vertical stratification. All hydrographic studies made during the bacteriological sampling periods should be described in detail.

Part 6. Bacteriological Data. This section should include summary data showing the bacteriological quality of water in tributary rivers or streams, sewage treatment plant effluent, and at the selected sampling points in the estuary. The section should also include data on the correlation of the E. C. presumptive test with the IMViC determinations.

Part 7. Conclusions. This section should include an interpretation of bacteriological findings in terms of their relationships to the observed sources of pollution and the hydrographic characteristics of the estuary.

Part 8. Appendix. The appendix should include all bacteriological results obtained on growing area water, shellfish, tributary streams, and treatment plant effluents.

In addition to an interim report for each area studied the State should also prepare a brief comparative review of the results obtained from the polluted area and the control area.

Appendix E

LIST OF PARTICIPANTS IN THE 1959 SHELLFISH SANITATION RESEARCH PLANNING CONFERENCE

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